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SEQUENCE LISTING

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NEW ENGLAND BIOLABS, INC.  
BOSTON BIOMEDICAL RESEARCH INSTITUTE

<120> METHOD FOR GENERATING SPLIT, NON-TRANSFERABLE GENES  
THAT ARE ABLE TO EXPRESS AN ACTIVE PROTEIN PRODUCT

<130> NEB-163-PCT

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<150> 60/135,677  
<151> 1999-05-24

<160> 134

<170> PatentIn Ver. 2.0

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2006250-03200



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&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Escherichia coli

&lt;400&gt; 9

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Glu Lys Leu Ser

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&lt;211&gt; 34

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&lt;210&gt; 12

&lt;211&gt; 32

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&lt;213&gt; Escherichia coli

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&lt;210&gt; 13

&lt;211&gt; 31

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&lt;213&gt; Escherichia coli

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<211> 38

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic,  
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<212> DNA

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based on Salmonella typhimurium

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<223> Description of Artificial Sequence: Synthetic,  
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&lt;213&gt; Artificial Sequence

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&lt;210&gt; 41

&lt;211&gt; 29

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic from  
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&lt;210&gt; 42

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Escherichia coli

&lt;400&gt; 42

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Val	His	Val	Asp	Ile	Asp	Pro	Ala	Glu	Ile	Gly	Lys	Asn	Lys	Gln	Pro
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His	Val	Ser	Ile	Cys	Ala	Asp	Val	Lys	Leu	Ala	Leu	Gln	Gly	Met	Asn
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Ala	Leu	Leu	Glu	Gly	Ser	Thr	Ser	Lys	Lys	Ser	Phe	Asp	Phe	Gly	Ser
65					70					75				80	

Trp	Asn	Asp	Glu	Leu	Asp	Gln	Gln	Lys	Arg	Glu	Phe	Pro	Leu	Gly	Tyr
			85					90						95	

Lys	Thr	Ser	Asn	Glu	Glu	Ile	Gln	Pro	Gln	Tyr	Ala	Ile	Gln	Val	Leu
			100					105					110		

Asp Glu Leu Thr Lys Gly Glu Ala Ile Ile Gly Thr Gly Val Gly Gln  
 115 120 125

His Gln Met Trp Ala Ala Gln Tyr Tyr Thr Tyr Lys Arg Pro Arg Gln  
 130 135 140

Trp Leu Ser Ser Ala Gly Leu Gly Ala Met Gly Phe Gly Leu Pro Ala  
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Ala Ala Gly Ala Ser Val Ala Asn Pro Gly Val Thr Val Val Asp Ile  
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Asp Gly

<210> 43

<211> 179

<212> PRT

<213> Escherichia coli

<400> 43

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 35 40 45

Pro His Val Ser Ile Cys Ala Asp Ile Lys Leu Ala Leu Gln Gly Leu  
 50 55 60

Asn Ser Ile Leu Glu Ser Lys Glu Gly Lys Leu Lys Leu Asp Phe Ser  
 65 70 75 80

Ala Trp Arg Gln Glu Leu Thr Glu Gln Lys Val Lys His Pro Leu Asn  
 85 90 95

Phe Lys Thr Phe Gly Asp Ala Ile Pro Pro Gln Tyr Ala Ile Gln Val  
 100 105 110

Leu Asp Glu Leu Thr Asn Gly Asn Ala Ile Ile Ser Thr Gly Val Gly  
 115 120 125

Gln His Gln Met Trp Ala Ala Gln Tyr Tyr Lys Tyr Arg Lys Pro Arg

130 135 140

Gln Trp Leu Thr Ser Gly Gly Leu Gly Ala Met Gly Phe Gly Leu Pro  
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Ala Ala Ile Gly Ala Ala Val Gly Arg Pro Asp Glu Val Val Val Asp  
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Ile Asp Gly

<210> 44  
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 <212> PRT  
 <213> Escherichia coli

<400> 44

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 1 5 10 15

Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys  
 20 25 30

Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Gln  
 35 40 45

Pro His Val Ser Ile Cys Ala Asp Ile Lys Leu Ala Leu Gln Gly Leu  
 50 55 60

Asn Ser Ile Leu Glu Ser Lys Glu Gly Lys Leu Lys Leu Asp Phe Ser  
 65 70 75 80

Ala Trp Arg Gln Glu Leu Thr Val Gln Lys Val Lys Tyr Pro Leu Asn  
 85 90 95

Phe Lys Thr Phe Gly Asp Ala Ile Pro Pro Gln Tyr Ala Ile Gln Val  
 100 105 110

Leu Asp Glu Leu Thr Asn Gly Ser Ala Ile Ile Ser Thr Gly Val Gly  
 115 120 125

Gln His Gln Met Trp Ala Ala Gln Tyr Tyr Lys Tyr Arg Lys Pro Arg  
 130 135 140

Gln Trp Leu Thr Ser Gly Gly Leu Gly Ala Met Gly Phe Gly Leu Pro  
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Ala Ala Ile Gly Ala Ala Val Gly Arg Pro Asp Glu Val Val Val Asp  
 165 170 175

Ile Asp Gly

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<212> PRT

<213> Escherichia coli

<400> 45

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 20 25 30

Val Leu His Ile Asp Ile Asp Pro Thr Ser Ile Ser Lys Thr Val Thr  
 35 40 45

Ala Asp Ile Pro Ile Val Gly Asp Ala Arg Gln Val Leu Glu Gln Met  
 50 55 60

Leu Glu Leu Leu Ser Gln Glu Ser Ala His Gln Pro Leu Asp Glu Ile  
 65 70 75 80

Arg Asp Trp Trp Gln Gln Ile Glu Gln Trp Arg Ala Arg Gln Cys Leu  
 85 90 95

Lys Tyr Asp Thr His Ser Glu Lys Ile Lys Pro Gln Ala Val Ile Glu  
 100 105 110

Thr Leu Trp Arg Leu Thr Lys Gly Asp Ala Tyr Val Thr Ser Asp Val  
 115 120 125

Gly Gln His Gln Met Phe Ala Ala Leu Tyr Tyr Pro Phe Asp Lys Pro  
 130 135 140

Arg Arg Trp Ile Asn Ser Gly Gly Leu Gly Thr Met Gly Phe Gly Leu  
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Pro Ala Ala Leu Gly Val Lys Met Ala Leu Pro Glu Glu Thr Val Val  
 165 170 175

Cys Val Thr Gly  
 180

05936500.022502

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 Val Ile His Met Asp Ile Asp Pro Ala Glu Met Asn Lys Leu Arg Gln  
 35 40 45  
 Ala His Val Ala Leu Gln Gly Asp Leu Asn Ala Leu Leu Pro Ala Leu  
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 Gln Gln Pro Leu Asn Gln Cys Asp Trp Gln Gln His Cys Ala Gln Leu  
 65 70 75 80  
 Arg Asp Glu His Ser Trp Arg Tyr Asp His Pro Gly Asp Ala Ile Tyr  
 85 90 95  
 Ala Pro Leu Leu Leu Lys Gln Leu Ser Asp Arg Lys Pro Ala Asp Cys  
 100 105 110  
 Val Val Thr Thr Asp Val Gly Gln His Gln Met Trp Ala Ala Gln His  
 115 120 125  
 Ile Ala His Thr Arg Pro Glu Asn Phe Ile Thr Ser Ser Gly Leu Gly  
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 Pro Asn Asp Thr Val Val Cys Ile Ser Gly  
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<212> DNA

<213> Escherichia coli

<400> 48

tgcggtcgac ttgcccgact accttggtga tctc 34

<210> 49

<211> 41

<212> DNA

<213> Escherichia coli

<400> 49

cccaagcttg gcgccatgag taaaggagaa gaacttttca c 41

<210> 50

<211> 36

<212> DNA

<213> Escherichia coli

<400> 50

gcgaccgggt tatttgtata gttcatccat gccatg 36

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<212> DNA

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<400> 52

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<211> 41

<212> DNA

<213> Escherichia coli

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41

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<212> DNA

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<400> 54

cagcgtagac ggcgccgtgg gatttggtta agcagtagc agc

43

<210> 55

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<213> Escherichia coli

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catgccatgg gggaagcggg gatcgccgaa g

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<210> 56

<211> 39

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<213> Escherichia coli

<400> 56

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39

<210> 57

<211> 34

<212> DNA

<213> Escherichia coli

<400> 57

cgaattctat ggttaaagtt atcggtagta gata

34

<210> 58

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<212> DNA

<213> Escherichia coli

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36

<210> 59

<211> 154

<212> DNA

<213> Nicotiana tabacum

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<213> Nicotiana tabacum

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<213> Nicotiana tabacum

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 England Biolabs, Inc.)

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<223> nucleotides 2493-5993: Nicotiana tabacum

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<223> Nucleotides 2493-6242: Nicotiana tabaceum

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<210> 65

<211> 42

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<213> *Nicotiana tabacum*

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<210> 78

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<212> PRT

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<210> 79

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Leu Phe Lys Gln Pro

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<210> 80

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Cys Leu Asn Ser Asp

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Cys Leu Asn Thr Asp

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<210> 83

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Val Phe Lys Gln Pro

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Cys Leu Asn Ser Met

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<210> 89

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Cys Leu Asn Asn Tyr

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Cys Leu Asn Ile Ile  
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Leu Phe Lys His Glu  
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<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 97

Val Phe Lys His Phe

1

5

&lt;210&gt; 98

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 98

Cys Leu Asn Ser Val

1

5

&lt;210&gt; 99

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 99

Val Phe Lys Gln Ile

1

5

&lt;210&gt; 100

&lt;211&gt; 5

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 100

Met Phe Lys Gln Ala

1

5

&lt;210&gt; 101

&lt;211&gt; 5

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 101

Leu Phe Lys His His

1 5

<210> 102

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 102

Leu Phe Lys His Gln

1 5

<210> 103

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 103

Met Phe Lys His Val

1 5

<210> 104

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon



<400> 104

Val Phe Lys Gln Lys  
1 5

<210> 105

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 105

Leu Phe Lys Gln Gln  
1 5

<210> 106

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 106

Leu Phe Lys His Ser  
1 5

<210> 107

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 107

Cys Leu Asn Thr Gly  
1 5

<210> 108

<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 108  
Cys Leu Asn Ser Arg  
1 5

<210> 109  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 109  
Val Phe Lys His Leu  
1 5

<210> 110  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 110  
Cys Leu Asn Asn Ile  
1 5

<210> 111  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the

Leu Phe Lys His Gln

1 5

<211> 5

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Cys Leu Asn Lys His

1 5

<211> 5

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Met Phe Lys Gln Tyr

1 5

<211> 5

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Cys Leu Asn Lys Gln

1 5

<210> 115

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 115

Cys Leu Asn Met Ser

1 5

<210> 116

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 116

Leu Cys Leu Asn Ile Leu Ala

1 5

<210> 117

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 117

Asn Cys Leu Asn Ile Asn Ala

1 5

<210> 118

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 118

Leu Met Phe Lys His Leu Ser

1

5

<210> 119

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 119

Thr Leu Phe Lys His Thr Arg

1

5

<210> 120

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 120

Lys Val Phe Lys Gln Lys Glu

1

5

<210> 121

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 121

His Leu Val Phe Lys His Leu

1

5

&lt;210&gt; 122

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 122

Leu Cys Leu Asn Thr Leu Leu

1

5

&lt;210&gt; 123

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 123

Leu Cys Leu Asn Asn Leu Val

1

5

&lt;210&gt; 124

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

&lt;400&gt; 124

Glu Val Phe Lys His Glu Gly

1

5

&lt;210&gt; 125

&lt;211&gt; 7

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 125

Lys Val Phe Lys Gln Lys Gly  
1 5

<210> 126

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 126

Thr Cys Leu Asn Thr Thr Ile  
1 5

<210> 127

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 127

Met Cys Leu Asn Asn Met Asn  
1 5

<210> 128

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

Leu Leu Phe Lys Gln Leu Arg  
1 5

<211> 7

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Arg Cys Leu Asn Asn Arg Leu  
1 5

<211> 7

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Met Val Phe Lys Gln Met Ala  
1 5

<211> 7

<212> PRT

<213> Artificial Sequence

**<220>**

<223> Description of Artificial Sequence: based on the ends of the Tn7 transposon

Ala Met Phe Lys Gln Ala Thr  
1 5

<210> 132

Figure 1 consists of 12 histograms arranged in a 4x3 grid, showing the distribution of the number of non-zero elements in the rows of the matrix  $A_k$  for  $k$  from 0 to 11. The x-axis for all histograms is 'Number of non-zero elements' ranging from 0 to 10. The y-axis is 'Frequency' ranging from 0 to 10. The distributions are as follows:

- $k=0$ : peak at 4 (frequency 10)
- $k=1$ : peak at 4 (frequency 10)
- $k=2$ : peak at 4 (frequency 10)
- $k=3$ : peak at 4 (frequency 10)
- $k=4$ : peak at 4 (frequency 10)
- $k=5$ : peak at 4 (frequency 10)
- $k=6$ : peak at 4 (frequency 10)
- $k=7$ : peak at 4 (frequency 10)
- $k=8$ : peak at 4 (frequency 10)
- $k=9$ : peak at 4 (frequency 10)
- $k=10$ : peak at 4 (frequency 10)
- $k=11$ : peak at 4 (frequency 10)



<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 132  
Leu Val Phe Lys His Leu Asp  
1 5

<210> 133  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 133  
Lys Met Phe Lys Gln Lys Thr  
1 5

<210> 134  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: based on the  
ends of the Tn7 transposon

<400> 134  
Tyr Cys Leu Asn Asn Tyr Phe  
1 5